

via the restriction recognition site NaeI. The sequence of pTC53 is shown in figure 4. For the insertion of the scFv-cDNA the Env-expression construct pTC53 was modified so that Sfi I and Not I specific restriction endonuclease recognition sites are inserted between the SNV-leader sequence and SNV-transmembrane sequence (TM) in a usual manner. For this purpose a recombinant PCR is carried out in a usual manner starting from the DNA of the plasmid PKA1558 (Scov. H. & Andersen, K.B., 1993) and the DNA coding of the anti-transferrin receptor scFv so that via Nru I (5' and 3') an insertion of the amplified fragment into the Nae I restricted pTC53 is possible. The thus inserted fragment contains the multiple Sfi I/Not I cloning site since the primers used further include a neighboring Sfi I or Not I recognition site, respectively, in addition to the terminal Nru I recognition site. For recombinant PCR, the following primers were used (SEQ ID NOs:32 and 33):¹

Please replace the paragraph beginning at page 12, line 22, with the following paragraph.

-- Preparation of zeocin resistance gene by means of PCR starting from DNA of the plasmid pSCV Zeo (Invitrogen Company): To select packaging cells after a stable transfection with the pTC53-zeo-scFv plasmid for a stable expression of the resistance gene, a zeocin cassette was integrated. For this purpose, a zeocin cassette was amplified by means of recombinant PCR from the vector pZeoSV2 (+/-) of Invitrogen Company (NV Leek, The Netherlands) and provided with the restriction sites NdeI 5' and 3' so that the cassette subsequently could be inserted into the NdeI restricted portion of the pUC19 backbone of pTC53. The PCR-batch (100 µl) contained: 1 x PCR buffer (Taq: 10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 10 µM (+)- and 10 µM (-)-primer, 200 µM of each deoxynucleotide, 2.5 units of Taq polymerase and 100 ng of plasmid DNA. The following oligonucleotides have been used (SEQ ID NOs:34 and 35):--

Please replace the Sequence Listing filed with the application with the paper copy of the Sequence Listing filed herewith.

Seq. List.
attached.
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